Supplemental data

Supplemental method

Study protocol

Fecal butyrate measurements

Thirty milligrams of frozen stool samples were suspended in 600 μL of 100% ethanol and homogenized by ultrasonication. After centrifugation, 10 μL of the supernatant was mixed with 10 μL of 1 ppm 2-ethylbutyric acid (Sigma-Aldrich, MO, USA) as an internal standard, and labeled with 2-nitrophenylhydrazide using a Short- and Long-Chain Fatty Acid Labeling Kit (YMC Co., Ltd. Kyoto, Japan). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was conducted using a high-performance liquid chromatography system coupled to a triple-quadrupole mass spectrometer (LC-MS-8040, Shimadzu Co. Kyoto, Japan). Chromatographic separation was performed at a flow rate of 350 μL/min and at 40 °C, using a 150×2.1-mm Mastro C18 3 μm (Shimadzu GLC Ltd. Kyoto, Japan) with a mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile). The separation gradient was as follows: 16% B at 0 min; 16 to 25% B in 6 min; 25 to 40% B in 3 min; and 40 to 95% B in 8 min. MS analyses were performed using electrospray ionization in the positive ion mode. The ions were detected using multiple reaction monitoring with mass transitions of *m*/*z* 196.05→43.1 for acetic acid; 210.1→57.15 for propionic acid; 224.1→71.305 for butyric and isobutyric acid; 238.1→85.05 for valeric and isovaleric acid; and 252.15→99.2 for 2-ethylbutyric acid. LC-MS/MS data were analyzed by using LabSolutions Insight software (Shimadzu Co.). Butyrate concentration was normalized to the wet fecal sample weight.

	Prevalence % (≥1 read)			
	Pre-HSCT		Day 28	
Species	Prebiotics	Control	Prebiotics	Control
Propionibacterium acidifaciens	0	1.4	0	2.8
Odoribacter splanchnicus	13.3	22.2	6.7	2.8
Porphyromonas asaccharolytica	0	0	0	0
Porphyromonas endodontalis	0	0	0	0
Porphyromonas gingivalis	0	0	0	0
Porphyromonas uenonis	0	0	0	0
Alistipes putredinis	6.7	22.2	3.3	11.1
Clostridium botulinum	0	0	0	0
Clostridium butyricum	0	0	0	0
Clostridium perfringens	0	4.2	6.7	0
Clostridium sporogenes	0	0	0	0
Anaerofustis stercorihominis	0	0	0	0
Eubacterium cellulosolvens	0	0	0	0
Eubacterium desmolans	0	0	0	0
Eubacterium hallii	0	5.6	3.3	2.8
Eubacterium limosum	10	12.5	0	0
Eubacterium rectale	16.7	6.9	3.3	0
Eubacterium saphenum	0	0	0	0

Eubacterium ventriosum	0	8.3	3.3	1.4
Pseudoramibacter alactolyticus	0	0	0	0
Anaerostipes caccae	0	0	0	0
Butyrivibrio crossotus	0	0	0	0
Butyrivibrio fibrisolvens	0	0	0	0
Butyrivibrio proteoclasticus	0	0	0	0
Clostridium saccharolyticum	0	0	0	0
*Clostridium symbiosum**	26.7	48.6	13.3	5.6
Coprococcus catus	0	9.7	0	2.8
Coprococcus comes	3.3	6.9	3.3	1.4
Coprococcus eutactus	0	1.4	0	0
Lachnoanaerobaculum saburreum	0	0	0	0
Roseburia hominis	0	6.9	3.3	1.4
Roseburia intestinalis	6.7	8.3	0	0
Roseburia inulinivorans	0	6.9	0	0
Shuttleworthia satelles	3.3	1.4	3.3	0
Anaerococcus hydrogenalis	0	0	3.3	0
Anaerococcus lactolyticus	0	0	0	0
Anaerococcus prevotii	0	0	0	0
Anaerococcus tetradius	0	0	0	0
Anaerococcus vaginalis	0	0	0	0

Peptoniphilus duerdenii	0	0	0	0
Peptoniphilus harei	0	0	0	0
Peptoniphilus lacrimalis	0	0	0	0
Eubacterium yurii	0	0	0	0
*Peptoclostridium difficile**	10	19.4	10	6.9
Anaerotruncus colihominis	16.7	22.2	3.3	2.8
*Faecalibacterium prausnitzii**	36.7	41.7	10	8.3
Subdoligranulum variabile	3.3	4.2	0	0
Eubacterium dolichum	0	0	0	0
Holdemanella biformis	0	0	0	0
Acidaminococcus fermentans	0	5.6	0	0
Acetonema longum	0	0	0	0
Megasphaera micronuciformis	6.7	4.2	23.3	0
Fusobacterium gonidiaformans	0	0	0	0
Fusobacterium mortiferum	20	9.7	3.3	5.6
*Fusobacterium nucleatum**	0	1.4	0	0
*Fusobacterium ulcerans**	13.3	20.8	20	9.7
Fusobacterium varium	0	0	0	0
Brachyspira murdochii	0	0	0	0
Brachyspira pilosicoli	0	0	0	0
Treponema phagedenis	0	0	0	0
Treponema vincentii	0	0	0	0
Total	76.7	83.3	56.7	29.2

Supplemental table 1. Butyrate-producing bacteria selected for analysis in the current study.

*: The following pairs of species were assigned the same bit score by glsearch:

Fusobacterium ulcerans/Fusobacterium sp. 12_1B Fusobacterium nucleatum/Fusobacterium sp. 3_1_36A2

Clostridium symbiosum/Clostridium sp. 7_3_54FAA

Faecalibacterium prausnitzii/Faecalibacterium sp. DJF_VR20

Peptoclostridium difficile/[Clostridium] difficile

		Prebiotics group (*n*=30)	Historical control group (*n*=72)	*P* value
Sex				0.14
	Male	17	44	
	Female	13	28	
Age				0.171
	<55	23	44	
	≥55	7	28	
Primary disease				0.826
	AML	13	32	
	ALL	7	12	
	MDS	4	14	
	Others	6	14	
Disease risk*				0.665
	Low risk	19	42	
	High risk	11	30	
Number of transplantation				0.629
	1	28	69	
	≥2	2	3	
Donor sex				0.36
	Male	22	45	
	Feale	8	27	
Sex match				0.14
	Female to male	2	14	
	Others	28	58	
HLA disparity (X6)				0.528
	0	15	17	
	1	6	18	
	2	6	7	
	≥3	3	10	
Allo-HSCT type				0.708
	rPBSCT	6	10	
	rBMT	1	2	
	uPBSCT	0	1	
	uBMT	13	42	

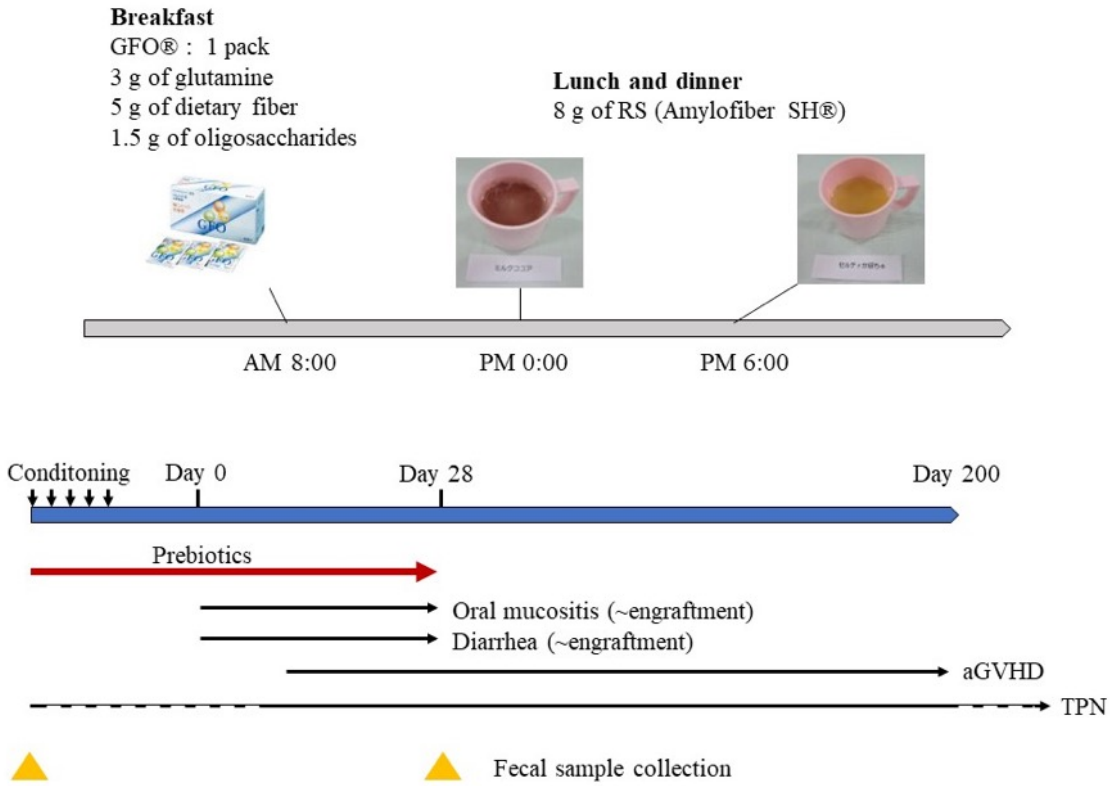
	CBT	4	7	
	Haplo (PT-CY)	3	3	
	Haplo (low dose ATG+ steroid)	3	7	
Conditioning				0.179
	MAC	22	41	
	RIC	8	31	
ATG				0.329
	Yes	5	7	
	No	25	65	
TBI				0.415
	Yes	26	56	
	No	4	16	
GVHD prophylaxis				0.419
	CsA based	7	12	
	FK based	23	60	
Use of antibiotics with relatively higher anaerobic activity (conditioning to day 28) †				1.0
	Yes	23	56	
	No	7	16	

Supplemental table 2. Patient characteristic (feces contributor, only)

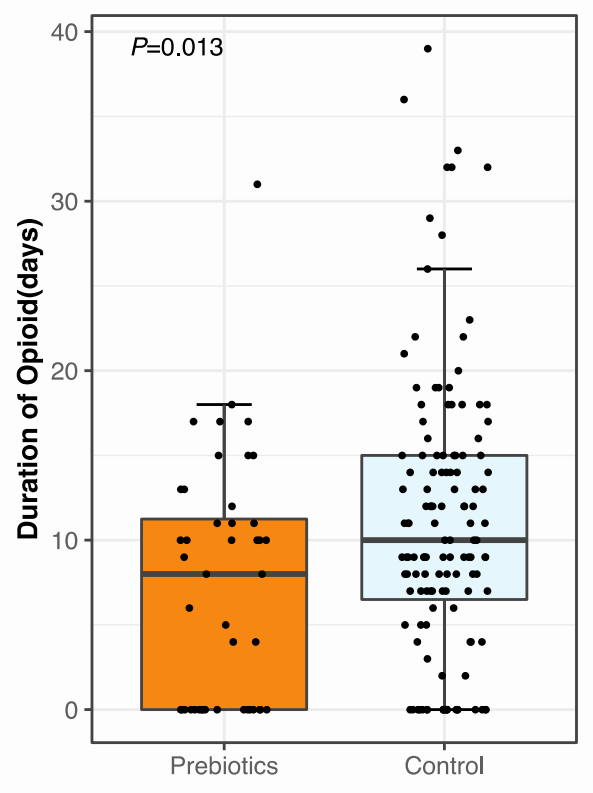
Abbreviations: ALL, acute lymphoblastic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BMT, bone marrow transplantation; CBT, cord blood transplantation; CsA, cyclosporine; FK, tacrolimus; GVHD, graft-versus-host disease; Haplo, haploidentical transplantation; HLA, human leukocyte antigen; MAC, myeloablative conditioing; MDS, myelodysplastic syndrome; PBSCT, peripheral blood stem cell transplantation; PT-CY, post-transplant cyclophosphamide; r, related; RIC, reduced-intensity conditioning; TBI, total body irradiation; u, unrelated.

*: Low disease risk included acute leukemia in first complete remission (CR), CML in first chronic phase, myelodysplastic syndromes in refractory anemia, malignant lymphoma in CR, nonmalignant hematologic diseases. All other diagnoses and 2nd allo-SCT were included in high disease risk.

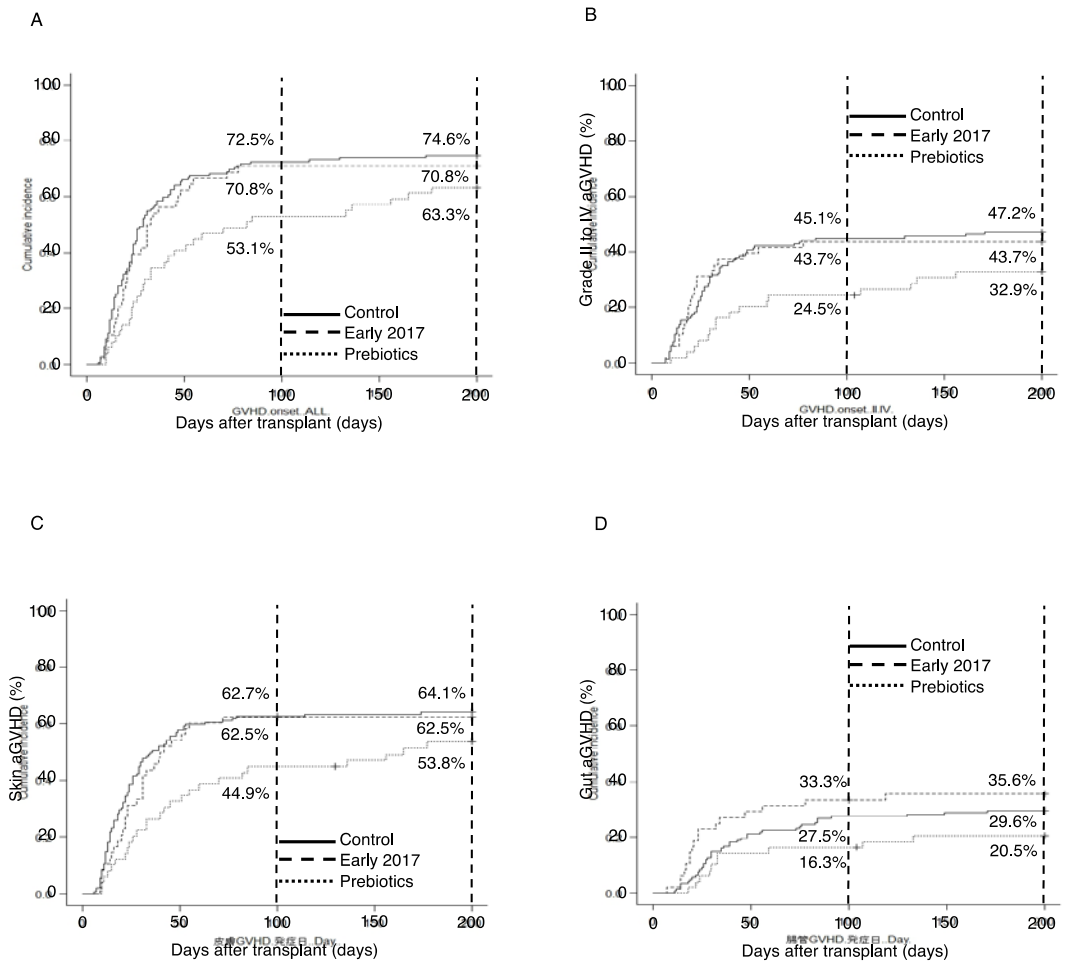
†: Antibiotics with relatively high anti-anaerobe activity: meropenem, imipenem-cilastatin, and piperacillin-tazobactam. The other antibiotics were third- and fourth-generation cephems, polypeptides, quinolones, sulfamethoxazole-trimethoprim, aminoglycosides, and aztreonam.



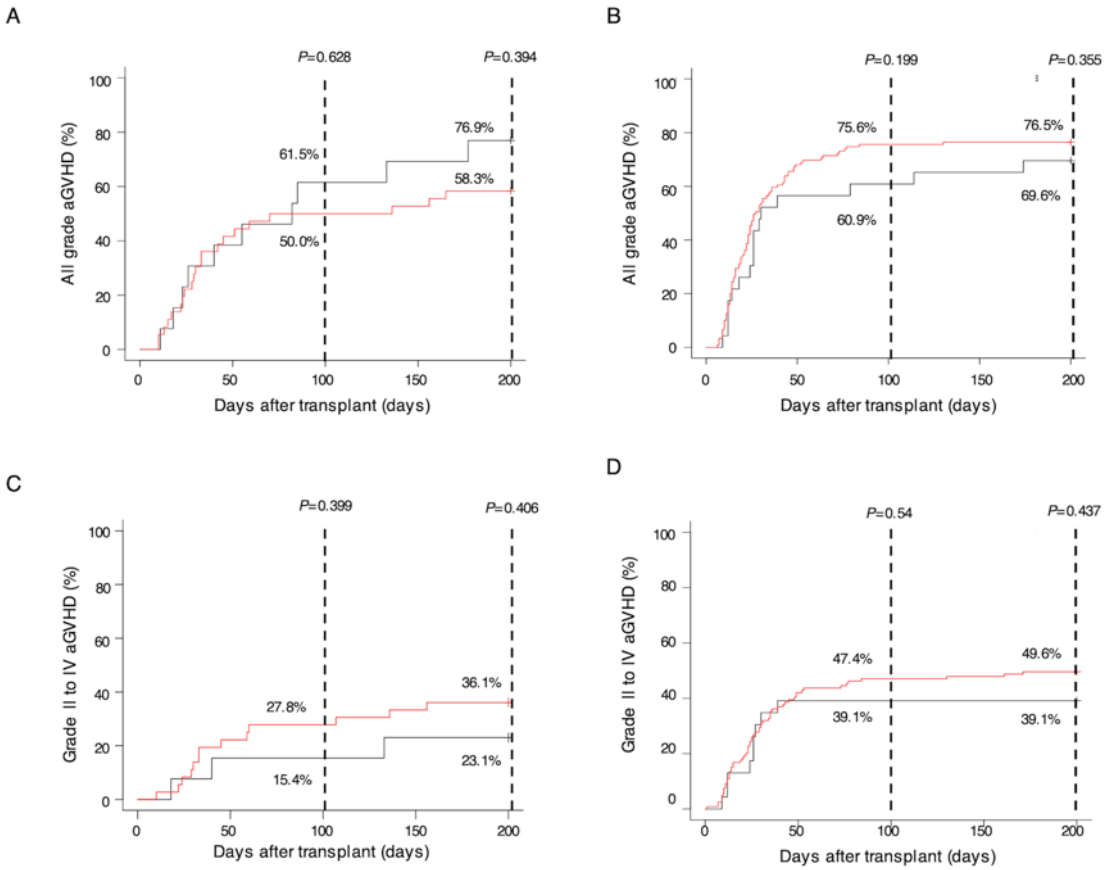
Supplemental figure 1. Study schema.



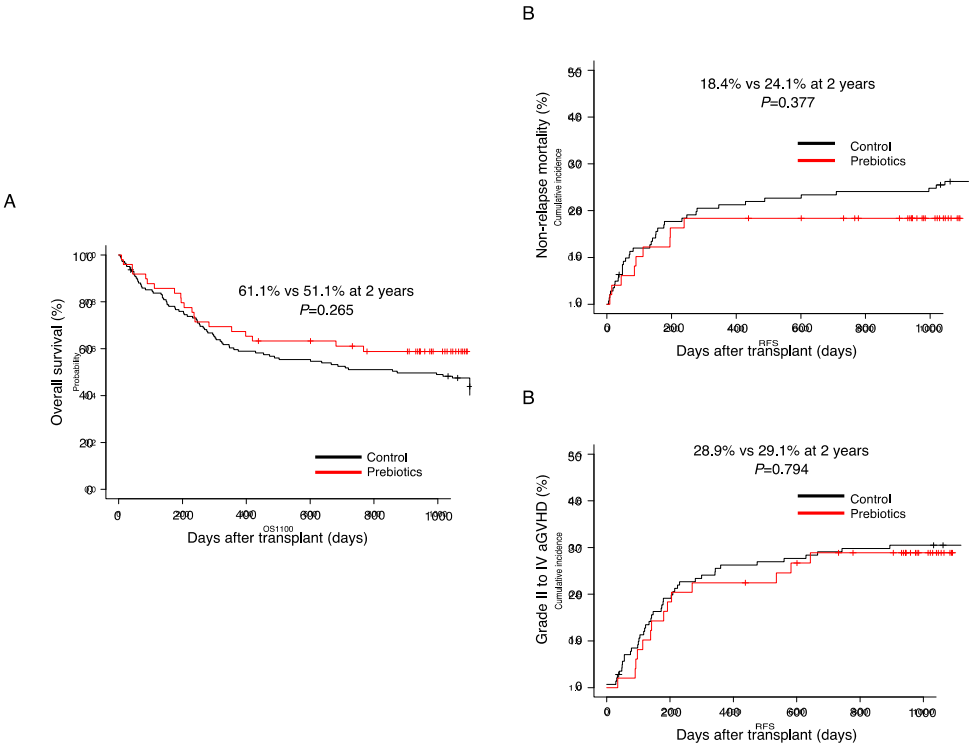
Supplemental figure 2. Duration of opioid use. Each dot represents each patient. The duration of opioid use until engraftment was significantly shorter in the prebiotics group (median 8 days in the prebiotics group vs. 10 days in the historical control group, *P*=0.013). Furthermore, the proportion of patients who did not receive opioid therapy was also higher in the prebiotics group (34.1% in the prebiotics group vs. 14.5% in the historical control group).



Supplemental figure 3. The cumulative incidence of aGVHD in three different time periods. The cumulative incidences of all grade (A), grade II – IV (B), skin all stage (C), and gut all stage (D) aGVHD were evaluated in patients who received allo-HSCT between 2013 and 2015 (Historical control group), at early 2017, and at late 2017 (Prebiotics group). The cumulative incidences of aGVHD in early 2017 showed similar results to those in the historical control group, not in the prebiotics group.



Supplemental figure 4. The cumulative incidence of aGVHD according to the use of antibiotics with relatively high anti-anaerobe activity. The cumulative incidences of all grade (A, C) and grade II – IV (B, D) aGVHD were evaluated between patients who did (red line) or did not (black line) received antibiotics with relatively high anti-anaerobe activity from conditioning to engraftment in the prebiotics group (A, B) and in the historical control group (C, D). The use of such antibiotics did not significantly increase the cumulative incidences of aGVHD.



Supplemental figure 5. Clinical outcomes in patients. No significant differences in the overall survival (61.1% vs. 51.1% at 2 years, *P*=0.265) (A), non-relapse mortality (18.4% vs. 24.1%, *P*=0.377) (B), and the cumulative incidence of relapse (28.9% vs. 29.1%, *P*=0.794) (C) were noted between the two groups.